DATA EVALUATION RECORD

STUDY 3

CHEM 129059 | Imidacloprid (NTN 33893) | §162-3

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 42256378
Fritz, R., and E. Hellpointner. 1991. Degradation of pesticides under anaerobic conditions in the system water/sediment: Imidacloprid, NTN 33893. Bayer AG Laboratory ID No. 1520205-5. Mobay Report No. 101346. Unpublished study performed by Bayer AG, Leverkusen-Bayerwerk, Germany, and submitted by Miles Inc. (formerly Mobay Corporation), Stilwell, KS.

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CONCLUSIONS:

Anaerobic Aquatic Metabolism

1. Study MRID #42256378 is acceptable and completely satisfies the anaerobic aquatic metabolism 162-3 data requirement for imidacloprid.

2. Imidacloprid degraded with a calculated first order half-life of 27 days in anaerobic (flooded plus nitrogen atmosphere) silt loam sediment that was incubated in the dark at 22 ± 1 C for 1 year. The major degradate was NTN 33823 which increased to a total maximum average of 66.0% of the applied radioactivity at 249 days posttreatment and was 64.0% at 358 days. NTN 33823 was detected at

maximum averages of 20.0% of the applied at 60 days posttreatment in the water fraction and 51.5% at 249 days in the sediment. [^{14}C]Residues associated with the water fraction of the sediment:water systems decreased from an average of 93.4% of the applied radioactivity at day 0 posttreatment to 18.0% at 358 days. Evolved $^{14}CO_2$ was detected at $\leq 0.2 \pm 0.2\%$ of the applied during the study. Unextracted [^{14}C]residues increased to an average of 22.6% of the applied radioactivity by 358 days posttreatment.

METHODOLOGY:

Samples (101 g wet weight) of sieved (2 mm) silt loam pond sediment (13.6% sand, 58.4% silt, 28.0% clay, 3.15% organic carbon, pH 6.89) were placed in 1-L flasks and flooded with uncharacterized pond water; the total volume of each sediment:water system was 500 mL. Each system was amended with 2.5 g saccharose, then the flasks were sealed and incubated (conditions not reported) for 30 days. Following the 30-day incubation, methylene-labeled [^{14}C]imidacloprid [NTN 33893; 1-((6-chloro-3-pyridinyl)methyl)-4,5-dihydro-N-nitro-1H-imidazol-2-amine; radiochemical purity >99%, specific activity 140 uCi/mg, Bayer AG], dissolved in water, was added to each sediment:water system at 0.56 ppm. Flasks were purged with nitrogen during and following treatment, then sealed with a closeable wash bottle attachment and incubated at 22 $\pm$ 1 C in darkness. Two flasks containing untreated sediment:water were incubated alongside the treated systems as controls. During the study, excess pressure generated by the sediment:water systems was periodically released into an air sample bag connected to the wash bottle attachment that sealed each flask (Appendices 1 and 2). Duplicate flasks were collected at 0, 3, 7, 14, 30, 60, 120, 249, and 358 days posttreatment. At each sampling interval, a 10-mL aliquot of volatiles in the headspace was sampled through a septum in the side of the flask using a gas-tight syringe and reserved for qualitative analysis. To quantitate remaining volatiles, each flask and attached air sample bag were purged with nitrogen (100 mL/minute); the nitrogen was then drawn through a trapping tower containing a layer of oil-covered quartz wool to trap organic volatiles followed by two layers of soda lime (separated by quartz wool) to trap CO_2, then finally through a copper oxide combustion apparatus to oxidize any additional organic volatiles (Appendices 7 and 8). Following collection of volatiles, the pH, redox potential, and dissolved oxygen content of the water phase in each system were determined; these parameters were also measured in the two control systems at each sampling interval.

Following sampling, a portion of the water phase was removed and aliquots were analyzed for total radioactivity using LSC. The remaining water fraction was decanted from the sediment and centrifuged. The supernatant was decanted from the sediment pellet which was rinsed with water and centrifuged again. The two supernatants were combined; an aliquot was acidified to release any dissolved $^{14}CO_2$, then analyzed for total radioactivity using LSC. For

characterization analyses, aliquots of the combined supernatant from water samples collected at 0, 3, 7, and 14 days posttreatment were analyzed directly, 30-day samples were concentrated prior to further analysis, and 120-day samples were applied to a Sep-Pak C-18 cartridge and eluted with methanol. Supernatants of 60-, 249-, and 358-day water samples were extracted four times with chloroform; organic phases were combined, then organic and aqueous phases were concentrated. Aliquots of water samples or extracts were analyzed by one-dimensional TLC on silica gel plates using ethyl acetate:2-propanol:water (65:23:12, v:v:v) and methylene chloride:methanol:-acetic acid:water (65:25:3.5:3.5, v:v:v:v). Radioactive areas were detected using a linear analyzer and autoradiography; it was reported that radioactive areas were quantified by manual evaluation, but the method was not described. Degradates were identified by comparison with unlabeled reference standards cochromatographed with the samples and visualized under UV light. Aliquots of selected samples were also analyzed by HPLC using UV (270 nm) and radioactivity detection on a reverse phase column with a gradient elution combining either water:phosphoric acid (1000:0.1, v:v) and acetonitrile:phosphoric acid (1000:0.1, v:v) or aqueous 0.001 M pentane sulfonic acid:-phosphoric acid (1000:0.6, v:v) and 0.001 M pentane sulfonic acid in acetonitrile:phosphoric acid (1000:0.6, v:v). Radioactive peaks were identified by either comparison to retention times of unlabeled reference standards detected with UV absorbance or fortification of samples with the unlabeled reference standards prior to chromatography. To isolate unknown [^{14}C]compounds, aliquots of selected samples were analyzed by automated multiple development TLC (AMD-TLC) using HPTLC silica gel plates developed with gradient solvent systems combining methanol and methylene chloride; detection of radioactive areas was not described. Parent imidacloprid and degradate identifications were confirmed using GC/MS, electron impact MS, and NMR.

Sediment was extracted sequentially three times with methanol, once with ethyl acetate, and finally once with methylene chloride. All extracts were separated from sediment by centrifugation and combined. The combined extract was concentrated, diluted with water, then partitioned three times with methylene chloride; organic phases were combined. Organic and aqueous phases were concentrated, then aliquots were analyzed for total radioactivity using LSC and additional aliquots were analyzed by TLC and HPLC as described above. To release bound [^{14}C]residues, subsamples of previously extracted sediment were dried, then refluxed for 6 hours with acetone:1 N HCl (75:25, v:v); aliquots of the extract were analyzed by LSC and TLC as described above. Extracted sediment was dried and milled, then unextracted [^{14}C]residues remaining in the sediment were quantified using LSC following combustion.

Oil-coated quartz wool from the trapping towers was extracted with ethyl acetate; an aliquot of the extract was analyzed for total radioactivity using LSC. Soda lime from the trapping towers was placed in a flask, then adsorbed $^{14}CO_2$ was released from the soda lime

using HCl and trapped in Carbosorb:Permafluor V solution (Appendix 9); the trapping solution was analyzed for total radioactivity using LSC.

To produce additional material for degradate characterizations, a large-scale experiment was performed. Silt loam sediment was placed in a 5-L glass bottle to a depth of approximately 1 cm, flooded (volume unspecified) with pond water, sealed, and pre-incubated for 30 days. The sediment:water system was then treated with methylene-labeled [^{14}C]imidacloprid at 6 ppm, sealed, and incubated at 22 $\pm$ 1 C in the dark. Samples of the water phase (volumes unspecified) were collected at 135, 186, 253, 347, and 407 days posttreatment; aliquots were analyzed by LSC and TLC as described above. Additional samples of the water phase were collected at 189, 267, and 413 days posttreatment; aliquots of the water fractions were either purified using a Sep-Pak C-18 cartridge or partitioned with methylene chloride. [^{14}C]Compounds were isolated using TLC and AMD-TLC techniques and analyzed using MS and NMR.

DATA SUMMARY:

Methylene-labeled [^{14}C]imidacloprid [NTN 33893; 1-((6-chloro-3-pyridinyl)-methyl)-4,5-dihydro-N-nitro-1H-imidazol-2-amine; radiochemical purity >99%], at 0.56 ppm, degraded with an observed half-life of between 30 and 60 days (27 days calc.) in anaerobic (flooding plus nitrogen atmosphere) silt loam sediment that was incubated in the dark at 22 $\pm$ 1 C for 358 days. Imidacloprid decreased from an average of 95.4-96.0% of the applied radioactivity (sediment plus water) at 0-7 days posttreatment to 67.0% at 30 days, 16.0% at 60 days, 4.7% at 120 days, and was $\leq$0.2% at 249 and 358 days (Appendix 17). The only degradate identified in the sediment and water fractions was

NTN 33823.

NTN 33823 increased to a total maximum average of 66.0% of the applied radioactivity at 249 days posttreatment and was 64.0% at 358 days; NTN 33823 was detected at maximum averages of 20.0% of the applied at 60 days posttreatment in the water fraction and 51.5% at 249 days in the sediment (Appendices 14 and 15). One unidentified "aqueous-soluble" unknown was detected in the water fraction at a maximum of 2.2 $\pm$ 0.2% (0.015 ppm) of the applied radioactivity, and two unknowns, one "organic-soluble" and the other "HCl/acetone-soluble", were detected in the sediment at maximums of 0.8 $\pm$ 0.1% (0.005 ppm) and 10.6 $\pm$ 0.4% (0.062 ppm) of the applied, respectively; from the information provided, it could not be determined if these unknowns were the same compound or different compounds. Unextracted [^{14}C]residues increased to an average of 22.6% of the applied radioactivity by 358 days posttreatment; evolved $^{14}CO_2$ was detected at $\leq$0.2 $\pm$ 0.2% of the applied during the study (Appendices 11 and 13). [^{14}C]Residues associated with the water fraction of the sediment:water systems decreased from an average of 93.4% of the

applied radioactivity at day 0 posttreatment to 18.0% at 358 days (Appendix 11). During the study, the dissolved oxygen content of the treated sediment:water systems was $\leq$2.8% of maximum oxygen saturation (Appendix 10). The pH of the treated systems ranged from 5.9 to 6.3 between 0 and 14 days posttreatment, then increased to 6.8 to 7.4 between 30 and 358 days. The redox potential of the treated systems decreased from 60-63 mV at day 0 posttreatment to -196 to -203 mV at 30 days, then ranged from -132 to -180 mV between 60 and 358 days. The dissolved oxygen content, pH, and redox potential values of the treated systems were comparable to those measured in control sediment:water systems. Material balances ranged from 94.0 $\pm$ 0.8 to 99.7 $\pm$ 2.0% of the applied (Appendix 11).

COMMENTS:

1. As only small amounts of the primary degradate NTN 33823 were released when the sediment was extracted sequentially with methanol, ethyl acetate, and methylene chloride, the extracted sediment was refluxed with acetone:1 N HCl for 6 hours to completely extract NTN 33823. The study authors reported that preliminary experiments showed that the extraction with acid under reflux did not degrade parent imidacloprid and that NTN 33823 was not an artifact of the procedure.

2. Using first order kinetics where x = time and y = ln(average concentration of imidacloprid as percent of applied radioactivity), the study authors calculated a degradation half-life of 27.12 days (Appendix 17); however, the data indicate that the pattern of degradation of the pesticide does not follow first order kinetics. No degradation of imidacloprid occurred from 0 to 7 days posttreatment, but the rate of degradation increased from 7 to 60 days (Figure in Appendix 17).

3. The study authors presented the average concentrations of NTN 33823 (as percent of applied radioactivity) detected in the water and sediment fractions at each sampling interval in Appendices 14 and 15. The Dynamac reviewer calculated the total average concentrations of NTN 33823 detected in the water plus sediment at each sampling interval using the data provided in those Appendices. Total average concentrations of parent imidacloprid in the water plus sediment at each sampling interval were provided by the study authors in Appendix 17.

4. The test sediment and water were collected from a pond located in Stanley, Kansas, but the length of time between collection and use of the sediment and water was not reported. Storage conditions of the sediment and water prior to use were not completely described; the study authors reported that the sediment and water were stored in 50-L glass aquaria under "field conditions" with forced aeration. The study authors should report what constitutes field conditions.

5. The pond water was not completely characterized; the total alkalinity, total hardness, and concentration of suspended solids were not reported. The dissolved oxygen content of the water fraction of the sediment:water systems was measured at each sampling interval, but was reported as a percentage of the maximum oxygen saturation in the water (= 8.73 mg O_2/L water at 22 C).

6. The silt loam classification of the sediment could not be confirmed because the sediment was not classified according to the USDA soil classification system. The particle size scale used was not equivalent to the USDA scale; the silt fraction contained sand and silt particles. The grain size distribution reported by the study authors was 13.6% sand (2000 - 63 um), 58.4% silt (63 - 2 um), and 28.0% clay (<2 um; Appendix 3). A CEC value for the sediment was not reported.

7. The study authors reported that water fractions were analyzed for solubilized $^{14}CO_2$; an aliquot of the sampled water phase was placed in a flask, treated with 5 N HCl, and stirred for 2 hours. Air was then drawn (600 mL/minute) through the flask into a trapping tower to collect volatiles and CO_2. Results from this procedure were not reported.

8. The study authors reported that to quantitate slightly soluble [$^{14}CO_2$]carbonates, subsamples of extracted 120- and 249-day sediment were dried, ground, placed in flasks, and treated with 5 N HCl:water (40:30, v:v); air was drawn through the flasks then through Carbosorb:Permafluor solution to trap released $^{14}CO_2$. Results from this procedure were not reported.

9. The study authors reported that five solvent systems were used for TLC; acetonitrile:water:25% ammonia (80:18:2, v:v:v), methylene chloride:methanol (90:10, v:v), ethyl acetate, ethyl acetate:2-propanol:water (65:23:12, v:v:v), and methylene chloride:methanol:-acetic acid:water (65:25:3.5:3.5, v:v:v:v). It was reported that the ethyl acetate:2-propanol:water and methylene chloride:methanol:acetic acid:water solvent systems were used to identify and quantitate imidacloprid and its degradates, but it was not specified what purposes the other three systems served. Following TLC, the study authors reported that radioactive areas were detected using a linear analyzer and quantified via manual evaluation; the manual evaluation method was not described. In addition, detection and identification of radioactive areas following AMD-TLC was not described.

10. The conditions for the batch study were not adequately described. The study authors reported that a 5-L glass bottle containing a 1-cm layer of sediment was filled up with pond water; the final volume of the sediment:water system should be reported. The sample sizes of water collected at the sampling intervals were not reported.

    It was unclear if results from the batch study were comparable to the definitive study. The study authors reported that the first water

samples for characterization analyses were not collected from the batch study until 189 days posttreatment because of "the increased rate and the resulting delayed degradation of the active ingredient." It was unclear if that statement meant that the initiation of degradation of imidacloprid in the batch study was even more delayed than in the definitive study and/or possibly related to the treatment rate; quantitative results were not provided from the batch study. Parent imidacloprid and NTN 33823 were isolated from the water fraction of the batch study and identifications were confirmed using MS and NMR. In addition, the degradate DIJ 9817 was isolated and identified; however, it was reported that DIJ 9817 was not detected in the definitive study.

11. The registrant should provide chemical names for all degradates of imidacloprid.

12. The registrant reported that imidacloprid [NTN 33893; 1-((6-chloro-3-pyridinyl)methyl)-4,5-dihydro-N-nitro-1H-imidazol-2-amine] is a broad spectrum, systemic insecticide. The proposed maximum use rates for food and non-food crop uses are 0.5 lb ai/A or 500-560 g/ha (Study 4, MRID 42256379). The study authors reported that a commercial use rate of 200 g/ha in a water layer of 10-cm depth would result in 0.2 ppm imidacloprid; therefore, an application rate of 0.6 ppm (0.28 mg [^{14}C]imidacloprid in 500 mL of sediment plus water) was chosen for this study to ensure identification of imidacloprid and possible degradates.

Pages 8-24 Access to FIFRA registration data is restricted under FIFRA section 10(g)